Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

A CHEMICAL ANALYSIS OF SUDAN GRASS SEED

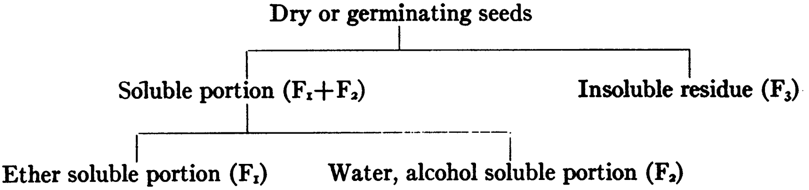
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 258

F. M. SCHERTZ

(WITH ONE FIGURE)

The method as here outlined was originally taken from the methods of WALDEMAR KOCH,[1] who employed it in the analysis of brain tissues. The method was then further modified by F. C. KOCH,[2] of the department of physiological chemistry, University of Chicago, where the work was chiefly on animal tissues. The method was again modified to meet the needs of plant tissues.

Outline of method


Dry or germinating seeds
Soluble portion (F_1+F_2)
Insoluble residue (F_3)
Ether soluble portion (F_1)
Water, alcohol soluble portion (F_2)

Fraction 1 (F_1) is the ether soluble portion; fraction 2 (F_2) is the portion soluble in alcohol or water; and fraction 3 (F_3) is the portion which is insoluble in ether, water, or alcohol. The dry seeds were ground finely before making the extraction, while the germinating seeds were ground in a mortar as finely as possible. The material was then placed in the extraction cups and extracted for 4 hours. A 1-hour extraction with ether was then made and the ether extract was added to the alcohol extract. The residue was dried, ground in a mortar, and then a water extraction was made. This water extraction and the residue was then made up to 70 per cent alcohol and again extracted with 95 per cent alcohol for 12 hours. In some cases this extraction was found to be insufficient, and consequently the extraction was prolonged for another 12 hours or more. The extraction was conducted at the boiling point of the solvent, using the KOCH extractor.

---

[1] KOCH, WALDEMAR, Methods for the quantitative chemical analysis of animal tissues. Archives Neurology and Psychiatry 4:11. 1909; also Jour. Amer. Chem. Soc. 31:1329–1364. 1909.

[2] Outline for the analysis of tissues as prepared by F. C. KOCH.

F_1 and F_2.—All of the alcohol, water, and ether extracts were added to each other, and then the whole was rapidly evaporated down to a thick syrup on the water bath. It was then transferred to a vacuum desiccator and dried until nearly a constant weight was obtained. This took from one to three weeks. The air in the desiccator was changed once or twice daily. This gave the weight of F_1 and F_2. The dry mixture of F_1 and F_2 was now extracted with anhydrous ether; this extract was F_1, and the residue was F_2. The evaporating dish plus F_2 was dried and again weighed, giving the weight of F_1 by difference, and also the weight of F_2. The ether extract F_1 was divided into two portions, one portion being used for the determination of sulphur and phosphorus, and the other for nitrogen. The residue was dissolved or suspended in 70 per cent alcohol and made up to a volume of 1000 cc. Of this, 50 cc. was used to determine the total sugars; 100 cc. for ash and for solids; 200 cc. for nitrogen; 100 cc. for free reducing sugars; and 550 cc. for sulphur and phosphorus.

F_3.—F_3 was then dried at 105° C. in an electric oven to nearly a constant weight. The whole was then pulverized thoroughly and fractions of it, ranging from 0.5 to 2.0 gm., were used for the determination of sulphur, phosphorus, nitrogen, total carbohydrates, ash, and crude fiber.

Moisture was obtained by difference. Nitrogen was estimated by means of the Kjeldahl method as modified by Gunning and Arnold. The nitrogen was multiplied by 6.25 to give the protein. Sulphur was estimated by the fusion ($Na_2CO_3+KNO_3$) method, precipitated, and weighed as $BaSO_4$. The filtrate from the sulphur determination was used and the phosphorus was determined from it by the Neumann-Pemberton method, by titration. Organic matter was determined by taking the weights of the ash F_2 and F_3 from the dry weights of F_2 and F_3 respectively. Sugars were estimated by the Bertrand volumetric method in connection with the Munson and Walker tables. Total reducing sugars were found by adding 10 cc. of HCl (sp. gr. 1.125) for every 100 cc.

of water used with the sample, and then boiling on a reflux condenser for 2.5 hours. They were then estimated as glucose.[3] Crude fiber was determined after the method in Bulletin no. 107, Bureau of Chemistry. 1912.

Analysis of unhulled dry seeds

The air dry weight of the seeds used in each case was 25 gm. The seed analyzed was that of Sudan grass (*Holcus halepensis sudanensis* [Piper] Hitchcock, or *Andropogon halepensis sudanensis* Piper). In each case two analyses were made and the results, together with the average of these two, are given in table I. The hulled seed was 70.62 per cent of the whole seed by weight, hence the hulls were 29.38 per cent of the whole seed by weight.

Analysis of seeds after germination

An analysis was made of the unhulled seeds which were kept in the refrigerator for 16 days at a temperature ranging from 8 to 20° C. A small percentage of the seeds showed signs of sprouting. In each case 25 gm. of seed were used.

This study was undertaken with the hope of discovering some of the early changes which take place on germination, and also because Sudan grass has promise as a forage grass. In comparing the unhulled dry seeds with the unhulled germinated seeds, it was found that the weight of F_1 remained constant, F_2 lost 2 per cent, and F_3 lost 3 per cent on germination. The protein in F_1 decreased, while that of F_2 increased somewhat. The total protein content of the germinated seeds increased about 1 per cent, due to the building of protein from the reserve substances. No change of importance was noted regarding the sulphur or phosphorus content. The ash of F_2 increased slightly at the expense of the ash of F_3. The amount of organic matter in F_2 decreased 1.5 per cent, while that of F_3 decreased 3 per cent; or a total loss of organic matter of about 5 per cent due to respiration. The greatest changes were found in the sugars. The total reducing sugar of F_2 decreased 2 per cent, free reducing sugar decreased slightly, and the total carbohydrates decreased about 9 per cent. The decrease in sugar-like products

---

[3] MATHEWS, ALBERT P., Physiological chemistry. 2d ed. New York. 1916.

TABLE I

	I	II	Average
Moisture		14.05	13.95
Weight of F_1		3.69	3.69
" " F_2		9.54	9.54
" " F_3	72.92	72.72	72.82
Total		100.00	100.00
	Protein*		
F_1		0.02	0.02
F_2		1.23	1.23
F_3	5.44	4.96	5.20
Total		6.20	6.45
	Sulphur		
F_1		0.02	0.02
F_2		0.05	0.05
F_3	0.07	0.09	0.08
Total		0.16	0.15
	Phosphorus		
F_1		0.004	0.004
F_2		0.06	0.06
F_3	0.20	0.24	0.22
Total		0.304	0.284
	Ash (inorganic matter)		
F_2		0.65	0.65
F_3	4.61	4.56	4.58
Total		5.21	5.23
	Organic matter		
F_2		8.89	8.89
F_3	68.31	68.16	68.24
Total		77.05	77.13
	Sugars		
F_2 total reducing		2.39	2.39
F_2 free reducing		0.96	0.96
F_3 carbohydrates	58.59	62.36	60.47
Total		64.75	62.86
	Crude fiber		
F_3	5.12	4.77	4.95

---

* The whole seed was analyzed and it gave a total for protein of 7.23 and 7.38 per cent.

TABLE II

RESULTS OF ANALYSIS OF HULLED DRY SEEDS*

	I	II	III	Average
Moisture	12.53	....	12.76	12.46
Weight of F_1	4.24	....	4.72	4.48
" " F_2	6.56	....	7.87	7.22
" " F_3	76.67	76.19	74.65	75.84
Total	100.00	....	100.00	100.00
	Proteins			
F_1	....	....	0.01	0.01
F_2	1.06	....	1.07	1.06
F_3	7.28	7.26	7.54	7.36
Total	....	....	8.62	8.43
	Sulphur			
F_1	0.01	....	0.01	0.01
F_2	0.05	....	0.08	0.06
F_3	0.27	0.21	0.17	0.22
Total	0.33	....	0.26	0.29
	Phosphorus			
F_1	....	....	0.002	0.002
F_2	0.08	....	0.10	0.09
F_3	0.26	0.22	0.25	0.24
Total	....	....	0.352	0.332
	Ash			
F_2	0.82	....	0.82	0.82
F_3	1.41	1.36	1.26	1.34
Total	2.23	....	2.08	2.16
	Organic matter			
F_2	5.74	....	7.05	6.40
F_3	75.26	74.83	73.39	74.50
Total	81.00	....	80.44	80.90
	Sugars			
F_2 total reducing	0.34	....	....	0.34
F_2 free reducing	0.27	....	0.26	0.27
F_3 carbohydrates	67.29	64.72	66.48	66.16
Total	67.63	....	....	66.50
	Crude fiber			
F_3	1.08	0.98	....	1.03

---

* Air dry weight of the seeds used in each case was 25 gm.

TABLE III

	I	II	Average
Moisture absorbed	48.22	45.72	46.97
Moisture	18.34	19.12	18.73
Weight of F_1	3.25	3.94	3.60
“ “ F_2	8.03	7.58	7.80
“ “ F_3	70.38	69.36	69.87
Total	100.00	100.00	100.00
		Proteins	
F_1	0.01	0.01	0.01
F_2	2.20	2.66	2.43
F_3	5.10	4.93	5.02
Total	7.31	7.60	7.46
		Sulphur	
F_1	....	0.01	0.01
F_2	0.05	0.05	0.05
F_3	0.16	0.13	0.15
Total	....	0.19	0.21
		Phosphorus	
F_1	0.002	0.001	0.001
F_2	0.06	0.08	0.07
F_3	0.21	0.23	0.22
Total	0.272	0.311	0.291
		Ash	
F_2	0.85	....	0.85
F_3	4.54	4.49	4.52
Total	5.39	....	5.36
		Organic matter	
F_2	7.19	....	7.19
F_3	65.84	64.87	65.36
Total	73.03	....	72.55
		Sugars	
F_2 total reducing	....	0.49	0.49
F_2 free reducing	0.40	0.33	0.37
F_3 carbohydrates	48.84	53.24	51.04
Total	....	53.73	51.53
		Crude fiber	
F_3	5.54	4.63	5.08

was about 11.5 per cent, due to respiration. Crude fiber remained practically constant.

When the hulled dry seeds were compared with the unhulled dry seeds, it was found that the weight of F_1 was 1 per cent greater in the former, and it was 2 per cent greater in the latter for F_2, while F_3 of the former was about 3 per cent greater. The proteins of F_1 and F_2 were about the same, but the protein of F_3 of the hulled dry seeds was more than 2 per cent greater. The ash of F_2 was slightly more in the hulled dry seeds, while the ash of F_3 was over 3 per cent greater in the unhulled seeds; hence a greater part of the ash was in the hulls. The organic matter of F_2 of the unhulled dry seeds was 2.5 per cent greater, while in F_3 it was 6 per cent less. The free reducing sugars were slightly greater in the unhulled seeds,

TABLE IV

UNHULLED DRY SEEDS

Material	I	II	Average
Free reducing sugars	1.10	0.93	1.02
Sucrose-like sugars	1.94	2.43	2.19
Total reducing sugars	3.32	2.71	3.02
Total carbohydrate F_3	60.00	59.60	59.80
Total	63.32	62.31	62.82*
	Unhulled seed grown at room temperature		
Free reducing sugars	1.02	0.96	0.99
Sucrose-like sugars	1.54	1.39	1.47
Total reducing sugars	3.24	3.25	3.24
Total carbohydrate F_3	43.36	45.88	44.62
Total	46.60	49.13	47.86
	Unhulled seed grown in refrigerator		
Free reducing sugars	0.89	0.75	0.82
Sucrose-like sugars	1.64	1.34	1.49
Total reducing sugars	2.69	2.69	2.69
Total carbohydrates F_3	43.51	43.93	43.72
Total	46.20	46.62	46.41

* Ten gm. of seed were hydrolyzed for 2.5 hours and gave a total carbohydrate of 65.30 per cent

the total reducing sugars were 2 per cent greater, while the carbohydrates were over 6 per cent less. Five times as much crude fiber was found in the unhulled seeds.

A further analysis of the sugars was then made. Two samples of 25 gm. each of the dry seed were analyzed for sugars alone. Two samples of 25 gm. each were grown at room temperature (16–24° C.) for 3 days, and two other samples were grown in the refrigerator for 32 days. The seeds in each case were extracted as indicated into the two portions F_2 and F_3. F_2 was then evaporated down and made up to a volume of 500 cc., of which 100 cc. was used for the determination of total reducing sugars; three 50 cc. samples for the inversion of cane sugar by weak hydrolysis at 67–69° C. for 10 minutes; and the remainder was used for free reducing sugars. All of the F_3 was hydrolyzed for 2.5 hours by adding 300 cc. water and 30 cc. hydrochloric acid (sp. gr. 1.125). From small portions of this the total sugars of F_3 were determined.

From table IV it is seen that when the seeds germinate the sucrose-like sugars decreased about 1 per cent, while there was a decrease in the total carbohydrates of about 15 per cent.

TABLE V

SUDAN GRASS COMPARED WITH OTHER SEEDS

Seeds	Water	Protein	Fat	N-free extract	Crude fiber	Ash	Sugar
Triticum sativum	13.37	10.93	1.65	70.01	2.12	1.92	2–7
Hordeum sativum	12.95	10.01	1.87	67.88	4.23	3.06	6–7
Secale cereale	13.37	11.19	1.68	69.36	2.16	2.24	2–3
Zea Mays	13.32	9–10	4–5	68–69	1.6–2.7	1.60	1.5–3.7
Sorghum saccharatum	14.58	9.44	3.18	68.55	2.54	1.71	....
Oryza sativa, hulled	13.17	8.13	1.27	75.50	0.88	1.03	1–2
Oryza sativa, unhulled	....	....	2.00	....	....	3.57	....
Avena sativa	12.8	10.25	5.27	59.68	9.97	3.02	2–5
Holcus halepensis sudanensis, unhulled	13.94	6.44	3.69	75.74*	4.95	5.24	2.39†
Holcus halepensis sudanensis, hulled	12.47	8.43	4.48	71.43*	1.03	2.16	3.44†
Unhulled germinated seed in refrigerator	18.72	7.46	3.60	59.78*	5.08	5.36	2.69†
Sudan grass seed (Kansas)‡	10.47	13.69	3.81	63.63	5.38	3.09	....

* 100 − (protein + ether extract + ash + moisture + crude fiber).

† Total reducing sugars as dextrose.

‡ THOMPSON, G. E., Sudan grass in Kansas. Kansas Agric. Exper. Sta. Bull. 212. 1916.

It is of interest to compare these results with those of some other workers. KJELDAHL, working on barley seed, found about 4.7 per cent cane sugar in the green malt and 1.1 per cent in the ungerminated barley. O'SULLIVAN found in ungerminated barley 0.8–1.6

and in malt 2.8–6.0 per cent cane sugar. These results on Sudan grass gave in each case less than 1.0 per cent of cane sugar, figuring the reducing sugar as cane sugar.

Compared with other grasses[4] it is very similar to *Sorghum avenaceum*, which gave the following results: ash 5.63, protein 3.29, cellulose 36.7, and fat 1.67 per cent. Of the ash, 1.5–3.0 per cent was CaO, P_2O_5, MgO, and SO_3.

Catalase activity

In each case 0.2 gm. (dry weight) of the seed was used. The results are given in cubic centimeters of oxygen set free in 10 minutes at 20° C.

Dry seeds		Seeds at room temperature 3 days
Hulled seeds	Unhulled seeds	Unhulled seeds
13.8	15.5	54.0
17.2	17.0	65.2
		68.2
15.0	16.25	
		62.4

Seeds in refrigerator 31 days	
Unhulled seeds	Unhulled seeds
45.0	46.0
50.8	50.6
45.6	50.0
47.1	49.0

The seeds which were grown in the refrigerator showed less catalase activity; part of this lessened activity may be due to the lowered temperature, but part of it undoubtedly was also due to the fact that the seeds at room temperature had grown slightly more than those in the refrigerator.

Microchemistry

A brief microchemical analysis was undertaken in order to locate the materials in the tissue of the seed, as well as to get an idea of how much was present (fig. 1).

Practically all of the cell walls gave the blue color reaction with 75 per cent H_2SO_4 and iodine, except the two small regions of the integument at each end of the caryopsis. With phloroglucin-HCl a cherry red color was observed in the pericarp integument near

[4] Wehmer, C., Die Pflanzenstoffe. Jena. 1911.

the micropylar end of the caryopsis. With acetone and a drop of concentrated HCl a red color was noted on the pedicel, and especially was the red prominent in the whole pericarp integument. This indicated strongly the presence of methyl pentosan, and perhaps araban and xylan. No callose was observed in any of the tissues. With ruthenium red, the pericarp integument and the cell membranes of the starchy endosperm gave slight tests, while the scutellum, plumule, plumule sheath, radicle, and root shoot gave a strong reaction, indicating the presence of much pectic substance. Small particles in the cells also gave a pectose reaction. The phloroglucin-HCl tests showed only traces of lignin, if any, present in the pedicel and in the glume. Upon heating the tissues with concentrated HNO_3 and concentrated $KClO_3$, ceric acid was observed to issue from the tissues of the pericarp integument. Suberin was present here.

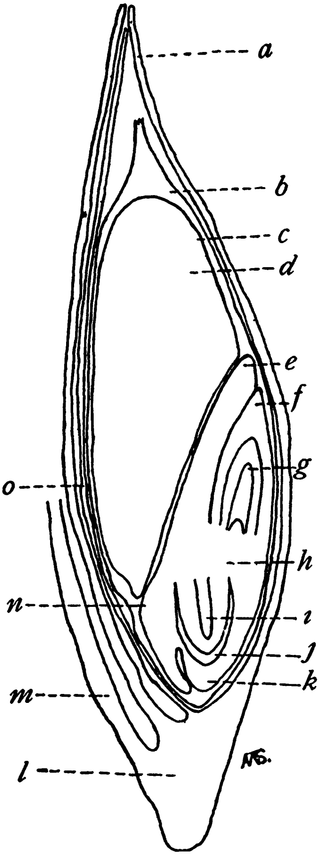


FIG. 1.—Longitudinal section of grain of Sudan grass: *a*, glume; *b*, pericarp; *c*, aleurone layer; *d*, endosperm; *e*, scutellum; *f*, coleoptile; *g*, plumule; *h*, embryo node; *i*, radicle; *j*, root cap; *k*, coleorhiza; *l*, pedicel; *m*, basal seta; *n*, glandular layer of scutellum; *o*, lodicule.

All cells of the embryo, and especially the cells of the embryo at the micropylar end, were rich in oil. The fat-containing cells of the endosperm stained heavily with Sudan III. Also, the epithelial layer had some fat present. The whole of the embryo became red when treated with concentrated H_2SO_4, and later took a greenish hue. Hence, phytosterol was thought to be present in the embryo, and also in a portion of the seed coat at the micropylar end of the caryopsis.

Silicon was found in the pericarp, as was shown by heating a dry section of the tissues with phenol. Tannins were found in the glumes and in the outer coats of the seeds, where red and purplish colors were observed, which were probably due to the oxidized tannins.

Two sizes of starch grains were found. The endosperm cells were filled with large sized starch grains, while the pericarp integument, the pedicel, and the basal seta had smaller grains in them.

Neither dextrin nor glucose was present in the embryo or in the endosperm, but considerable was present in the hulls. Amylodextrin was found in all of the endosperm cells in rather large quantities. The layers of the cells of the caryopsis outside of the fat-containing endosperm cells all gave a positive reaction for glucose when treated with copper tartrate and sodium hydroxide.

TABLE VI

MICROCHEMISTRY OF SUDAN GRASS SEED

Part of seed	Cellulose	Pentoses	Pectic substances	Lignin	Suberin	Fat	Phytosterol	Tannin	Starch	Glucose	Amylo-dextrin
Pedicel	+*	+	.....	+	.....	.....	.....	.....	+	.....	.....
Glume	+	.....	.....	+	.....	.....	.....	+	.....	++	.....
Basal seta	.....	.....	.....	.....	.....	.....	.....	.....	+	.....	.....
Lodicule	+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Pericarp	+	++	+	.....	+	.....	+	+	+	+	.....
Aleurone layer	.....	.....	.....	.....	.....	++	.....	.....	.....	.....	.....
Endosperm	+	.....	+	.....	.....	.....	.....	.....	++	.....	++
Epithelial layer	.....	.....	.....	.....	.....	+	.....	.....	.....	.....	.....
Scutellum	+	.....	++	.....	.....	+	+	.....	.....	.....	.....
Embryo node	+	.....	++	.....	.....	+	+	.....	.....	.....	.....
Radicle	+	.....	++	.....	.....	++	+	.....	.....	.....	.....
Coleorhiza	+	.....	++	.....	.....	++	+	.....	.....	.....	.....
Plumule	+	.....	++	.....	.....	+	+	.....	.....	.....	.....
Coleoptile	+	.....	++	.....	.....	+	+	.....	.....	.....	.....

* + = present; ++ = present in large amount.

In conclusion, I wish to acknowledge my obligations to Professor WILLIAM CROCKER, under whom this work was done, for his advice and valuable criticisms; to Dr. S. H. ECKERSON for her untiring interest and advice relative to the microchemistry; and to Professor F. C. KOCH for his helpful suggestions in the methods of chemical determinations involved.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.